OPTICAL DETECTION SYSTEM

FIELD OF THE INVENTION

The present invention is related to optical detection systems. In particular, the present invention is related to optical detection systems which can analyze multiple samples simultaneously.

BACKGROUND OF THE INVENTION

On many occasions, in chemistry and biology, large numbers of samples need to be analyzed. Particularly in molecular biology, in the Human Genome Project, high speed analyses with high throughput are necessary to achieve the goals of the project. Genetic mapping and DNA sequencing on slab gels are currently performed by using automated DNA sequencing with mono-colour or multi-color fluorescent dye labeling. Because capillary electrophoresis (CE) and particularly CE combined with laser induced fluorescence (CE-LIF) offers rapid charged species analyte separation and high detection sensitivity, it is particularly attractive as a separation technique in DNA sequencing applications. However, the number of capillaries that can be analyzed at one time limits the total throughput of the analysis. To increase the throughput a technique called capillary array electrophoresis (CAE) has been introduced. In this technique multiple capillaries are used in parallel with some advantages over slab gels with multiple lanes. There is a substantial reduction on Joule heating effect. Therefore, higher electric fields can be applied and faster analysis can be obtained. The cost of material is reduced in terms of gel usage due to the reduced diameter of the capillaries, as well as samples usage due to a smaller sample size. Another advantage is the

possibility to increase the sample throughput by increasing the number of channels in theory up to thousands, while the slab gels impose physical size and sample loading difficulties.

Various methods for acquiring signals from multiple channels have been described; however, the simultaneous detection of the different channels in CAE still presents some problems. A multiple capillary electrophoresis laser induced fluorescence detector that utilizes a confocal fluorescence scanner is described in US Pat. Nos. 5,091,652 and 5,274,240. The scanner or computer controlled stage translates the capillary array past the light path of a laser beam and the optical detection system. Since relatively heavy components are being moved problems with misalignments of the capillaries relative to the light source are likely to occur. To avoid problems derived from the movement of bulky components in US Patent No. 5675155, a detection system is described where an excitation laser beam is focused and scanned across the capillary array by the movement of a mirror which is aligned as well to receive the electromagnetic radiation from the sample. The advantages of these

Another multiplexed detector system for capillary electrophoresis is described in US Pat. No. 5,498,324. The invention involves laser irradiation of the sample in a plurality of capillaries through individual optic fibers inserted into the outflow of each capillary. Quesada and Zhang (Electrophoresis 17, 1841-1851, 1996) improved this design by using fiber optics for illumination and collection of the fluorescent emission orthogonally. One of the advantages of the this approach is that no moving parts are involved. However, in both systems the excitation energy that reaches each capillary does not have a homogeneous distribution and degrades as the number of fibers included in the fused taper splitter increases. In addition, detection of the arrays is simultaneous through a CCD combined with microscope or camera lens. Therefore, in this case the limitation of the number of capillaries that can be detected at one time depends on the number of them that can be packed in the imaging field of the detector and the resolution of the detector. The most critical problem in this approach may be cross talk between capillaries because fluorescence from adjacent capillaries can be refracted to reach the detector. Although cross talk between capillaries can be avoided by the use of spacers, it is evident that the use of spacers will reduce the number of capillaries in the array.

A greater number of capillaries can be measured at the same time (US Pat. No. 5730850) by arranging capillaries two-dimensionally in a capillary array sheet and using a simultaneous two-dimensional detector. Employing modified sheath-flow cuvette detection, sensitivity is enhanced by eliminating light interferences. However, the simultaneous illumination of all the capillaries requires a complicated system of mirrors to transmit the light beam

through the buffer solution path between the capillary holder and the detection window, which in turn may result in differences in intensity.

Accordingly, it is desirable to provide an economical and high sensitive detection system for multiple sample analysis which is easy to set up and easy to handle where bulky moving parts and complicated alignments are minimized.

OBJECT OF THE INVENTION

It is therefore an object of the present invention to provide a system to overcome the shortcomings as stated above.

It is another object to provide an optical detection system which allows only collimated light to reach the sample to be detected.

It is a further object to provide an embodiment of an optical detection system which can perform simultaneous detection in a plurality of samples with reduced scattering and cross-talk.

SUMMARY OF THE INVENTION

The present invention is an optical detection system comprising an electromagnetic radiation source, a source radiation focusing and collimating means, a photodetector, an emitted radiation focusing means and a source radiation blocking panel. The radiation source is used to direct source radiation onto a sample which is disposed in a sample platform. The source radiation focusing and collimating means is disposed between the radiation source and the sample for focusing and collimating the source radiation onto the sample. The photodetector is adapted for receiving radiation emitted from the sample which has been focused by the emitted radiation focusing means. The source radiation blocking panel, disposed between the source radiation focusing and collimating means and the sample, is unique in that it is capable of reducing light scattering and interference, such that a clear signal from each individual sample can be obtained by the photodetector.

In the most preferred embodiment, the source radiation focusing and collimating means comprises at least one convergent cylindrical rectangular lens and the source radiation blocking panel comprises a light absorbing panel with at least one pinhole. The samples are contained in channels or tubes aligned in parallel. For simplicity, the samples contained in the various channels or tubes are referred to as sample volumes. In one embodiment, the emitted radiation focusing means is a convex lens, while in another embodiment, it is a convergent cylindrical lens together with an emitted radiation blocking panel having pinholes. This panel with pinholes will be referred to simply as pinholes in the following description. The pinholes may be connected to scanning or conveying means to allow movement. The

system may be used for the detection of radiation absorbance or for fluorescence, including epi-fluorescence.Static pinholes for reducing interference and moving pinholes for sequentially and repetitively illuminating selected sample volumes from an array of samples. In the cases of no cross talk between samples or when cross talk can be eliminated, static pinholes are used to reduce interference due to scattered light, while moving pinholes can be used to eliminate cross talk between samples by sequentially and selectively illuminating only the sample volumes to be measured at any instant of time. In this embodiment, the system includes a plurality of sample volumes in parallel comprising: an array of channels, capillaries, flow cells, bands or wells; at least one electromagnetic radiation source; at least one convergent rectangular cylindrical lens to focus electromagnetic radiation; at least one set of static pinholes or moving pinholes; a scanner for moving the pinholes; and at least one detector aligned to receive electromagnetic radiation collected from the sample volumes. The pinholes are placed in between the array of samples and the detector and /or between the array of samples and the electromagnetic radiation source. For operation of the system using static pinholes, the number of pinholes should match that of the samples in the array. The electromagnetic radiation energy that reaches each sample volume is homogeneously focused and distributed by the convergent rectangular cylindrical lens through the array of pinholes. Emitted electromagnetic radiation from all of the sample volumes is collected and directed to a detector simultaneously. Pinholes are used to prevent scattered electromagnetic radiation from reaching the detector. In operation of the system using moving pinholes, the number of pinholes is less than that of the

samples in the array and can be as few as one. Only the electromagnetic radiation energy that can pass through the pinholes can reach selected sample volumes. The scanner for moving the pinholes adjusts the position of the pinholes so that only selected sample volumes are illuminated by the electromagnetic radiation. Emitted electromagnetic radiation from the selected sample volumes is collected and directed to a detector where a signal is generated in response to the interaction of the electromagnetic radiation with the sample. This operation is performed sequentially and repetitively with each sample volume in the array. Moving pinholes are also used to prevent scattered electromagnetic radiation from reaching the detector. Advantageously, the present invention provides two detection systems for multiple sample analysis, which are easy to set up and easy to handle wher bulky moving parts and complicated alignments are minimized, and allows the electromagnetic radiation source to remain on selected sample volumes for a pre-set period of time. The result is higher sample throughput, improved detection sensitivity and more economical and physically stable detection systems.

In preferred embodiments, the sample is imaged from an array of channels microfabricated in glass, quartz, fused silica or polymeric materials for capillary electrophoresis. In one embodiment, the source radiation is an excitation light, and the the sample in each channel is fluorescent or contains a fluorescent label and is separated on an electrophoretic medium, or the sample is not fluorescent and is separated in a fluorescent electrophoretic medium. The electromagnetic radiation source preferred is a laser but other light sources, mercury lamps, xenon lamps or any other light sources with the

appropriated power and wavelength can be used. The source radiation wavelength specific to the sample to be investigated is isolated by interference filter and transmitted axially to the sample. The source radiation is focused linearly by a convergent rectangular cylindrical lens. The focal distance between the lens and the channels is adjusted manually by movement of a translational stage in the x, y and z directions or by an auto-focusing system. In the same direction the fluorescent emission is collected and collimated by the lens through the array of pinholes or by moving pinholes. A long pass filter is selected to block wavelengths below the emission. An array of pinholes or moving pinholes can be used to prevent non-collimated light from reaching the detector.

The present invention provides detection systems with which a plurality of sample volumes can be analyzed. In consequence, this system allows for a significant increase in throughput of batches of samples. Different types of optical detection systems can be used, such as visible, ultraviolet or fluorescent. In the preferred embodiments, we will refer to CE-LIF (laser induced fluorescence), because of its higher sensitivity, performed in microfabricated channels. For those skilled in the art, it is well known that the system is equally applicable for capillary electrophoresis in fused silica capillaries, and since this system is provided with a focusing facility, any coplanar , linear and closely distributed samples can be easily incorporated into the field of view and optically analyzed by the detector. In addition, this multichannel detection system can be used in the analysis of chemicals, such as ions and drugs, or bio-molecules, such as DNA, RNA, proteins, viruses, bacteria and the like by HPLC or other analytical techniques

involving the use of capillaries, microchannels, flow cells, bands or wells. In general it can be useful for optical testing of series of homologous samples volumes distributed closely in reservoirs and in the same plane.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1A is a schematic representation of a embodiment of the multichannel detection system in microchip-based capillary electrophoresis utilizing a single moving pinhole for multiple epi-fluorescence detection.

Fig. 1B is a top view of filter wheel shown in Figure 1A.

Fig. 2 is the schematic layout of an array of 300 channels scanned simultaneously by 6 pinholes.

Fig. 3A is the schematic representation of an alternative embodiment of the multichannel detection system utilizing parallel multiple pinholes for multichannel multicolour fluorescence detection imaged on an array detector, e.g. a CCD camera.

Fig, 3B is top view of the filter wheel shown in Figure 3A.

Fig. 4. is the schematic representation of an alternative embodiment of the multichannel detection system utilizing an array of static pinholes for multiple epi-fluorescence detection.

Fig. 5. is the schematic representation of an alternative embodiment of the epi-fluorescence multichannel detection system utilizing an array of static pinholes for multicolour fluorescence detection.

Fig. 6. is a schematic representation of a preferred embodiment of the multichannel detection system in microchip-based capillary electrophoresis utilizing an array of static pinholes for simultaneous absorbance detection.

DESCRIPTION OF THE INVENTION

FIG. 1 is a general schematic illustration of the multichannel epifluorescent detection system using a moving pinhole. The system includes a radiation source **10**,an interference filter **11**, a dichroic beamsplitter **12**, a convergent cylindrical rectangular lens **13**, a long pass filter **14** and a photon detector **16**. The source irradiates excitation light **19** to the dichroic beamsplitter **12** which is positioned at an angle (which is 45^0 in this example) to the beam. This beamsplitter reflects radiation of wavelengths below the specified wavelength, acting as a long pass filter. The reflected radiation is then directed axially to the sample channels **20**. An interference filter **11** is preferably included in this embodiment to isolate the wavelength necessary for excitation of the fluorescent sample and at the same time eliminate the background scatter caused by the radiation of undesired wavelengths. The interference filter **11** is particularly essential to isolate the necessary excitation wavelength when the light source employed is not monochromatic, such as Hg, Xe, or tungsten lamps. The axis of the convergent cylindrical rectangular lens **13** is placed perpendicular to the microchannels **20** or, perpendicular to the array of samples to be determined. A single pinhole **17** with an aperture matching the size of the area to be detected allows the excitation beam to reach a selected sample. The resulting fluorescent emission **23** is collected axially by the convergent cylindrical rectangular lens **13**, and transmitted through the dichroic beamsplitter **12** and a long pass

scanner or conveyer system **21** causes the pinhole **17** (not drawn to size) to move to the next microchannel. In this manner, by scanning the pinhole **17**, the excitation radiation and the fluorescent emission is sequentially brought to and collected from every microchannel or sample volume in the array. The permanence time of the pinhole in every sample is pre-set and electronically controlled to allow for the excitation and emission of every individual sample before moving to the next. By incorporating a moving pinhole **17**, the detection system of the present invention avoids the interference caused by cross talk between channels since one sample is illuminated at the time. By using a pinhole **17**, interferences due to scattered light from the optics and the mass of the glass plate **22** comprising the channels are further avoided. The system can be modified for multicolour fluorescence detection by adding a rotating filter wheel **30** (shown in Figure 1B) before the detector. The filter wheel comprises a predetermined number (usually 4) of band filters which are designed to block the radiation at the wavelengths of the excitation radiation sources and transmit fluorescence at wavelengths typically longer than those for the excitation wavelengths. The filter wheel **30**, contolled by means of a rotor **26**, rotates and brings sequentially the set of filtered into the path of the emission beam, thus permitting the detection of the fluorescent emission of different dyes present in the sample.

Larger number of samples can be monitored by scanning a set of pinholes placed in series provided that the distance between them is large enough to avoid cross-talk between different channels. This situation is depicted in FIG. 2 where 300 microchannels **20** (as numbered as C1-C300) can be scanned by a set of 6 pinholes **27** simultaneously. This approach is advantageous

over individual channel scanning since the scanning time per cycle can be decreased. Therefore, the number of channels in the array can be increased. The optical signals collected by the photodetector may be further amplified by an amplifier (not shown in Fig.1) and analyzed by a computer **28**.

Although the preferred embodiment is to irradiate the sample and collect fluorescent emission in the same direction, another possibility is to irradiate the sample and collect fluorescent emission at a different angle. This angle can be varied as long as the excitation radiation **19** does not interfere with the emission radiation **23**.

FIG. 3A is a schematic diagram of a multi-wavelength fluorescence detection system for multichannel electrophoresis where a detector **30**, which may consist of several individual photodetectors, a multi-segmented photodetector

detection, the fluorescent emission **40** of each position is filtered though two band pass filters arranged in parallel in a filter wheel **42** as shown in FIG 3B. The advantage of using two (or more) laser lines isolated spatially is that a higher duty cycle can be realized compared to the use of filter wheels. With the improved sensitivity and throughput by using an array of moving pinholes, this system can be very useful in analyzing large number of samples.

In cases where there is no possibility of cross talk, or when cross talk can be effectively avoided, for example when channels are formed on opague materials, the use of an array of static pinholes is advantageous since no moving parts are involved. FIG. 4 is the general schematic illustration of simultaneous excitation and detection of an array of samples by using an array of static pinholes **44**. The system includes a radiation source **46**, an interference filter **48**, a dichroic beamsplitter **50**, a convergent cylindrical rectangular lens **52**, an array of pinholes 44, a long pass filter **56**, a second convergent cylindrical rectangular lens **58** and a photon detector **60**. The emitted fluorescent radiation **61** is collected in the same direction by the first convergent cylindrical rectangular lens **52** and transmitted through the dichroic beamsplitter **50** and a band pass filter **56**. A second convergent cylindrical rectangular lens **58** is placed in such direction that it collects, collimates and focuses linearly on to a photo-detector **60** the emitted fluorescent radiation **61** of every sample simultaneously. The array of pinholes **44** with an aperture of the size corresponding to the detection area is placed in front of the channels to avoid interference caused by scattered light from the optics and the mass of the glass plate **62** comprising the channels 64. A second array of pinholes **66** is placed before the photon-

detector in order to block any scattered non-parallel light from reaching the detector This signal may then be amplified by an amplifier **67**, and analyzed or stored by a computer **69**. Since fluorescence is emitted by the sample molecules in all directions, fluorescent refraction from neighboring channels can cause interferences in the detection. To avoid this cross-talk between channels, it is advisable to intercalate a set of blocking channels between pairs of separation channels. The blocking channels may be formed by filling channels with black ink to absorb unwanted fluorescent radiation or reflective materials to reflect radiation.

Although the preferred embodiment is to irradiate the sample and collect fluorescent emission in the same direction, another possibility is to irradiate the sample and collect fluorescent emission at a different angle. This angle can be varied as long as the excitation radiation **68** does not interfere with emission radiation **61**.

FIG 5 illustrates a schematic diagram of the multi-detector system with static pinholes suitable for the detection of two different emission wavelengths. This system makes use of a single laser source **70** to generate excitation light **72** of two different wavelengths for two different fluorescent labels. The two different fluorescent labels may be found within each sample inside each sample channel **73** in the sample platform **75**. These fluorescent labels should have readily distinguishable fluorescent emissions. During operation, the emission radiation of the fluorescent labels passes through an array of pinholes **77** and is collected by a convergent cylindrical rectangular lens **74**, refracted through a dichroic beam splitter **76** and split into two different wavelengths by the use of a second dichroic beam splitter **78**. Additional

spectral filtering is performed by using a band pass filter **80** for the lower wavelength and a long pass filter **82** for the higher wavelength. The fluorescent signals are then focused through an array of pinholes **84** by convergent cylindrical rectangular lenses **86** on to two photodetectors **88**. Again, the signals may be amplified by one or more amplifiers **85** and **87**, and the signal analyzed and stored by a computer **89**. Those skilled in the art will recognize that a higher number of fluorescent wavelengths can be detected by subsequent division and filtering of the fluorescent emission provided that the appropriated number of labels and excitation radiation wavelengths are used.

Another viable application for an array static pinholes is the detection in parallel of the absorbance of an array of samples volumes. As represented in FIG.6, the radiation source **90** and the photodetector **92** are place in the same plane. The source radiation is electromagnetic radiation, and the emitted radiation is the transmitted radiation which is not absorbed by the sample. Electromagnetic radiation **94** is focused linearly to the array of samples by a convergent cylindrical rectangular lens **96.** After the electromagnetic radiation **94** has pass through the sample volumes, the transmitted radiation **98** is collimated by a second convergent cylindrical rectangular lens **100**. A third convergent cylindrical rectangular lens **102** focuses on to the photodetector **92** the transmitted radiation. An array of pinholes is placed between the cylindrical lens **96** and the detection volume, allowing a parallel beam of light to pass through the sample. Another array of pinholes **94** is placed in front of the photodetector **92** to avoid scattered light from reaching the detector. As in the other systems, an amplifier **106** may be

connected to the photodetector to amplify the signal, The decrease in intensity of the electromagnetic radiation can then be calculated by the electronic components by connecting a computer **108** to the amplifier. This embodiment of the invention is expected to be most useful for detection in high performance liquid chromatography (HPLC), capillary HPLC or microchannel HPLC.

For microchannel separations, the channels **20, 64, 73** and **104** are microchannels which are generated in glass, quartz or fused silica plates **22, 62, 75** and **110** by photolithographic and standard dry or wet-etching techniques. Polymeric materials can also be molded to adopt the desired patterns. Materials which are transparent, physically and chemically stable such as polymethylmethacrylate, polydimethoxysilosane, nylon, polyethylene, polypropylene, fluoropolymers-based polymers and the like can be used as substrate for microfabrication. Microstructures for capillary electrophoresis comprise a channel network that permits dead-volume-free sample introduction and separation. In addition, other procedures such as sample pre-treatment, derivatization, fraction collection etc. can be integrated in the same microstructure. Arrays of microchannels are easily generated in the same structure. Each channel comprises a separation channel and injection channel. Typically, separation channels are straight or serpentine like of 1-100 cm length having a width of 1- 100 μm and a height in the order of 1-50 μm, and injection channels intersecting the separation channels. Voltages are applied at the end of the microchannels where buffer and sample reservoirs are located. Flow direction and separation can be controlled by electrokinetic

effects due to the harmonic application of voltages in the reservoirs or hydrodynamic effects due to application of pressure or vacuum.

A sample plug introduced into the separation channel is electrophoretically separated along the length of the separation channel. Monitoring of the separated components can be performed at the desired point along the separation channels by focusing the excitation beam and collecting the subsequent fluorescent emission. In this embodiment, the sample platform is a channel plate or a capillary array electrophoresis chip.

A channel plates may be placed in a translational stage to facilitate focusing of the sample volumes on the field of view by the movement of the stage in y, x and z directions. Focusing on the point of detection may be accomplished visually through a rotational trinocular that allows visualization of the channels. This facility is preferred since different microfabricated plates layouts, shapes and sizes can be incorporated and brought into focus. This procedure can be automated if x and z positions are fixed, and focused on the y direction is performed by a manual focusing device or an autofocusing device.

This invention is not limited to the above described details and pictorially accompanying drawings since many changes and modifications may be made to the invention without departing from the spirit and the scope thereof.